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Circulating Cytomegalic Endothelial Cells Are Associated With High Human Cytomegalovirus (HCMV) Load in AIDS Patients With Late-Stage Disseminated HCMV Disease

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The prevalence of circulating cytomegalic endothelial cells, detected currently by the pp65-antigenemia assay and described previously in blood of transplanted and AIDS patients with disseminated human cytomegalovirus (HCMV) infection, was found to be 2.9% in the AIDS population and 6.5% in the fraction of the AIDS population with HCMV in blood. Cytomegalic endothelial cells increased to 39.7% and 48.4%, respectively, in AIDS patients with very high levels of antigenemia and viremia, while an end organ disease reached an incidence of 76.4%. Positive and negative predictive values of cytomegalic endothelial cell detection for diagnosis of HCMV end organ disease were 73.1% and 21.4% with antigenemia levels >1,000, respectively. On the other hand, in a selected group of 38 cytomegalic endothelial cell-positive AIDS patients with <50 CD4+ T cells/μl and late-stage HCMV disease, who were followed-up for variable periods of time, the prevalence of high level antigenemia was 95.3%, that of viremia 86.0% and that of L-DNAemia 92.7%, while the incidence of HCMV end organ disease was 84.2%. In this population, it was shown that cytomegalic endothelial cell presence was associated with lack of (56.0% of episodes) or insufficient (4.0%) anti-HCMV treatment or emergence of HCMV drug-resistant strains (17.3%) or short-term response to antiviral treatment (22.7%); was determined in the same patient by different conditions during follow-up. Longitudinal observations indicated that cytomegalic endothelial cells were detected often in blood at least 3 months later than end organ disease suggesting that the duration of end organ disease was a cofactor associated with the appearance of cytomegalic endothelial cells. ***J. Med. Virol. 55:64–74, 1998.***

KEY WORDS: HCMV end organ disease; pp65-antigenemia; HCMV viremia; HCMV leukoDNAemia; HCMV drug-resistant strains; antiviral treatment

INTRODUCTION

In immunosuppressed and AIDS patients with disseminated human cytomegalovirus (HCMV) infection, HCMV inclusion bearing endothelial cells are often detected in multiple organs [Myerson et al., 1984; Roberts et al., 1989; Goodgame, 1993]. In 1993, two groups of researchers reported the presence of circulating cytomegalic cells, which appeared to be endothelial in origin, in blood of immunosuppressed patients with HCMV disease [Grefte et al.; Percivalle et al., 1993]. In addition, it was shown that these circulating cytomegalic endothelial cells were likely to originate from HCMV-infected endothelial cells disseminated throughout the vascular tree of multiple organs in patients with disseminated HCMV infection. These cells become progressively enlarged, detach from the blood vessel wall and enter the blood stream [Percivalle et al., 1993]. Furthermore, circulating cytomegalic endothelial cells were associated with high antigenemia levels, disappearing when antigenemia levels fell following antiviral treatment [Gerna et al., 1993; Grefte et al., 1993].

In the last 3 years, prophylactic approaches to HCMV infections in transplanted patients have nearly abolished the presence of cytomegalic endothelial cells

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in this group of patients, whereas cytomegalic endothelial cells continue to be detected in AIDS patients.

The incidence of cytomegalic endothelial cells in the AIDS population was studied. In addition, we investigated the following issues in a group of cytomegalic endothelial cell-positive AIDS patients: i) the association of cytomegalic endothelial cells levels with levels of HCMV pp65-antigenemia, viremia, leukoDNAemia (L-DNAemia), plasmaDNAemia (P-DNAemia) and end organ disease; ii) the clinical conditions causing the appearance of cytomegalic endothelial cell; iii) long-term follow-up of some AIDS patients aimed at monitoring the appearance of cytomegalic endothelial cell during the course of HCMV infection and during specific antiviral treatment; and iv) the short-term effect of antiviral therapy on levels of circulating cytomegalic endothelial cells during the first 2 weeks of treatment.

PATIENTS AND METHODS

Patients

The AIDS patients examined for HCMV infection in blood in our laboratory in 1994–1995 (1,099 patients and 1,888 buffy coat samples) were investigated for the prevalence of circulating cytomegalic endothelial cells. Some of these patients were examined on one occasion. In addition, 38 cytomegalic endothelial cell-positive AIDS patients with HCMV disease for >3 months were selected mostly from the same AIDS population, examined prospectively and included in the present study. All these patients, who had a $CD4^+$ cell count <50/µl and underwent clinical and virological follow-up for HCMV infection in blood for a varying period of time from 5 days to 24 months, were selected based on the detection of at least one cytomegalic endothelial cell episode. A cytomegalic endothelial cell episode was defined as the detection of one or more circulating cytomegalic endothelial cells in a blood sample drawn from an AIDS patient during the virological follow-up for HCMV infection. Patients undergoing antiviral treatment received either ganciclovir (5 mg/kg/12 hr) or foscarnet (90 mg/kg/12 hr) as induction treatment. Half the dosage of either drug administered once a day represented standard maintenance treatment. Suboptimal maintenance regimen (mostly due to drug toxicity) was represented by the administration of either drug at half a dosage 2–3 day/week.

Disseminated HCMV disease (in the absence of overt organ localization) was diagnosed by the presence of fever and/or thrombocytopenia and/or leukopenia associated with a high viral load in blood. For these patients (n = 6) HCMV-related symptoms are shown in Table I as "Fever". For HCMV end organ disease, diagnostic criteria followed the essential recommendations made by participants in the Workshop on HCMV disease [Ljungman and Plotkin., 1995]. Twenty-one patients had HCMV retinitis, 3 polyradiculomyelitis, 4 gastrointestinal disease, 2 pneumonitis, 2 encephalitis, 1 laryngitis and 1 hepatitis (2 patients had 2 sequential or simultaneous different organ syndromes, respectively). Quantitation of HCMV in blood was achieved by sequential determination of pp65-antigenemia, viremia, and both L-DNAemia and P-DNAemia.

Quantitation of pp65-Antigenemia and Circulating Cytomegalic Endothelial Cells

pp65-antigenemia was quantitated by fluorescence microscopy by counting the number of peripheral blood leukocytes (PBL) positive for pp65/2×10^5 cells examined on cytospin preparations stained with a pool of 3 pp65-specific monoclonal antibodies, according to Gerna et al. [1992b]. PBL were stained routinely from buffy coat samples. The number of circulating cytomegalic endothelial cells was determined in parallel on the same cytospin preparations stained with pp65-specific monoclonal antibodies and was expressed as the number of cytomegalic endothelial cells/2×10^5 PBL examined. Thus, if present, cytomegalic endothelial cells were counted in parallel with pp65-positive PBL. The identification of the two cell populations carrying virus or virus material was easy due to cell size, which was much smaller in pp65-positive PBL (either polymorphonuclear leukocytes or monocyte/macrophages), and the staining pattern, which was exclusively nuclear in PBL and predominantly (or exclusively) cytoplasmic in cytomegalic endothelial cells (Fig. 1). Ficoll separation of the mononuclear cell fraction was undertaken occasionally to increase cytomegalic endothelial cells number in cytospin preparations [Grefte et al., 1993; Percivalle et al., 1993]. However, the results reported in this paper were not obtained according to this procedure. Initially, the endothelial origin of cytomegalic endothelial cells was confirmed by using the same procedure and reagents reported previously [Percivalle et al., 1993].

Viremia, Virus Isolation and Identification

Quantitation of viremia was achieved by inoculating 2×10^5 PBL onto human embryonic lung fibroblast cell cultures by the shell vial technique and then counting the number of fibroblast nuclei positive for HCMV immediate-early antigen p72, 16–24 hr postinfection (pi) [Gerna et al., 1990]. Conventional virus isolation from PBL or other clinical samples was carried out on human embryonic lung fibroblast cell cultures after the appearance of cytopathic effects within 2 weeks pi. Negative cultures were passaged blindly for another 2 week period. HCMV was identified as reported previously [Gerna et al., 1990].

L- and P-DNAemia

DNA extraction from PBL and plasma samples was carried out according to the silica procedure [Boom et al., 1990] as previously reported [Gerna et al., 1994a]. Similarly, the PCR method for viral DNA quantitation with external standards and an internal amplification control was described previously [Gerna et al., 1994a]. Some modifications to the method were introduced in order to improve sensitivity and a new internal control was constructed following the same principle described previously [Zipeto et al., 1993]. The HCMV IE1 target sequences of the outer set of primers of a nested PCR protocol [Revello et al., 1995] were added to a pGEM 4Z

TABLE I. Quantitation of Human Cytomegalovirus (HCMV) in Blood of 38 AIDS Patients with Disseminated HCMV Infection and Presence of Circulating Cytomegalic Endothelial Cells (CEC) with Respect to Clinical Symptoms, Antiviral Treatment and Conditions for CEC Appearance

Patient number (time of follow-up, number of CEC episodes)	Day of follow-up	HCMV quantitation[a]				HCMV-related symptoms/syndrome	Antiviral treatment[b]		Condition for CEC appearance[c]
		Ag	Vir	CEC	L-DNAa/P-DNA number GE		GCV	PFA	
1 (13 m, 2)	391	1,000	26	5	50,000/1,794	Retinitis	I	—	GCV–Resistance
2 (40 d, 3)	2	730	290	8	83,176/ND	Polyradiculomyelitis	—	—	NT
3 (24 m, 5)	525	250	139	1	42,178/4,970	Laryngitis	SM	—	SD
	596	1,000	45	3	19,952/750		I	—	GCV–Resistance
	719	1,300	117	13	524,807/5,000	GID	—	I	PFA–Resistance (+GCV–Resistance)
4 (4 m, 3)	1	400	450	3	ND/ND	Retinits	—	—	NT
	96	1,000	36	14	ND/10,000		M	—	GCV–Resistance
5 (9 m, 2)	246	350	34	2	51,286/447	Retinitis	—	—	NT
6 (15 d, 2)	1	3,000	270	6	137,153/11,220	Polyradiculomyelitis	—	—	NT
7 (21 m, 2)	612	3,000	250	3	31,622/7,940	Retinitis	—	—	NT
8 (4 m, 1)	91	221	20	6	8,128/22	GID, Retinitis	—	SM	SD
9 (16 m, 1)	423	115	10	1	8,912/630	Retinitis	—	—	NT
10 (2 m, 2)	1	600	103	1	1,258/ND	Fever	—	—	NT
11 (7 m, 1)	139	2,000	10	3	7,943/44	Retinitis	I	—	GCV–Response
12 (45 d, 1)	1	700	119	1	1,199/ND	GID	—	—	NT
13 (20 d, 4)	1	1,000	600	1	50,118/3,981	Retinitis	—	—	NT
14 (25 d, 2)	1	900	57	1	63,095/12,590	Polyradiculomyelitis	—	—	NT
15 (15 d, 2)	3	500	26	1	5,689/251	Retinitis	—	—	NT
16 (2 m, 2)	1	185	16	3	2,511/446	Fever	—	—	NT
17 (40 d, 2)	16	385	13	4	10,000/501	Encephalitis	—	—	NT
18 (2 m, 2)	13	251	10	1	1,412/5[d]	Fever	—	—	NT
19 (14 m, 6)	347	3,000	1,000	1	12,302/7,943	Retinitis	I	—	GCV–Resistance
	360	3,000	300	3	52,480/3,467		—	I	PFA–Response
	406	3,000	400	9	1,584/1,995		—	—	NT
20 (4 m, 1)	1	90	25	1	2,510/ND	Retinitis	—	—	NT
21 (20 d, 1)	1	110	28	5	35,400/ND	Retinitis	—	—	NT
22 (20 d, 1)	1	54	1	1	446/ND	Retinitis	—	—	NT
23 (20 d, 1)	1	1,800	120	2	75,857/ND	Retinitis	—	—	NT
24 (20 d, 1)	1	118	3	1	707/ND	Retinitis	—	—	NT
25 (4 m, 2)	1	900	80	2	18,000/ND	Retinitis	—	—	NT
26 (20 d, 1)	1	115	40	1	18,700/ND	Retinitis	—	—	NT
27 (20 d, 1)	1	146	20	4	1,412/ND	Retinitis	—	—	NT
28 (20 d, 1)	1	1,500	400	5	199,000/ND	Retinitis	—	—	NT
29 (5 m, 5)	47	2,000	1,000	1	17,780/3,480	GID	—	I	PFA–Response
30 (5 m, 1)	1	276	40	3	28,180/ND	Retinitis	—	—	NT
31 (5 m, 1)	122	1,000	6	1	1,995/1,584	Fever, hepatitis	—	—	NT
32 (9 m, 4)	184	400	60	1	15,848/125	Fever	—	—	NT
33 (5 d, 2)	4	2,000	1	2	60,255/30,199	Fever, Pneumonitis	I	—	GCV–Response
34 (5 m, 2)	100	5,000	130	25	148,977/63,095	Fever, Pneumonitis	—	—	NT
35 (7 m, 1)	99	280	12	1	2,630/5[d]	Fever	—	—	NT
36 (5 m, 1)	97	150	0	1	501/5[d]	Fever	—	—	NT
37 (13 m, 2)	369	2,500	1,000	2	44,000/3,548	Encephalitis	—	—	NT
38 (7 m, 1)	205	455	5	1	21,379/3,801	Retinitis	—	—	NT

[a]Ag, pp65-antigenemia (number pp65-positive/2 × 10^5 PBL examined); Vir, viremia (number p72-positive culture fibroblasts/2 × 10^5 PBL inoculated into a shell vial); L-DNA, leukoDNAemia (number HCMV genome equivalents/1 × 10^5 PBL examined); P-DNA, plasmaDNAemia (no. HCMV genome equivalents/10 μL plasma); GE, genome equivalents.
[b]GCV, ganciclovir; PFA, foscarnet; I, induction; —, no drug.
[c]GCV-Resistant, ganciclovir-resistance; NT, no treatment; SM, submaintenance; SD, subdosage; GID, gastrointestinal disease; PFA-Resistance, foscarnet-resistance; M, maintenance; GCV-Response, ganciclovir-response; PFA-Response, foscarnet-response.
[d]Samples positive for DNA only by nested PCR were assigned an arbitrary number of 5 GE.

plasmid (Promega, Madison, Wis.) fragment generating a recombinant molecule. This molecule was amplified by the same set of primers in the same thermal conditions and with efficiency comparable to HCMV IE1 amplification, but the PCR products were different in size. The recombinant molecule, named pAC2, was cloned in PCR2000 plasmid (TA cloning kit, Invitrogen, San Diego, CA) and utilized as internal control of amplification. Similarly, HCMV IE1 PCR product, named pCM2, was cloned in PCR2000 and was used as external standard. HCMV DNA quantification was obtained as described [Gerna et al., 1994a, 1994b] by densitometric analysis of pAC2 and pCM2 specific signal after 3% agarose gel electrophoresis and ethidium bromide staining. Since pAC2 consisted of an heterologous DNA sequence flanked by HCMV E1 outer primers target

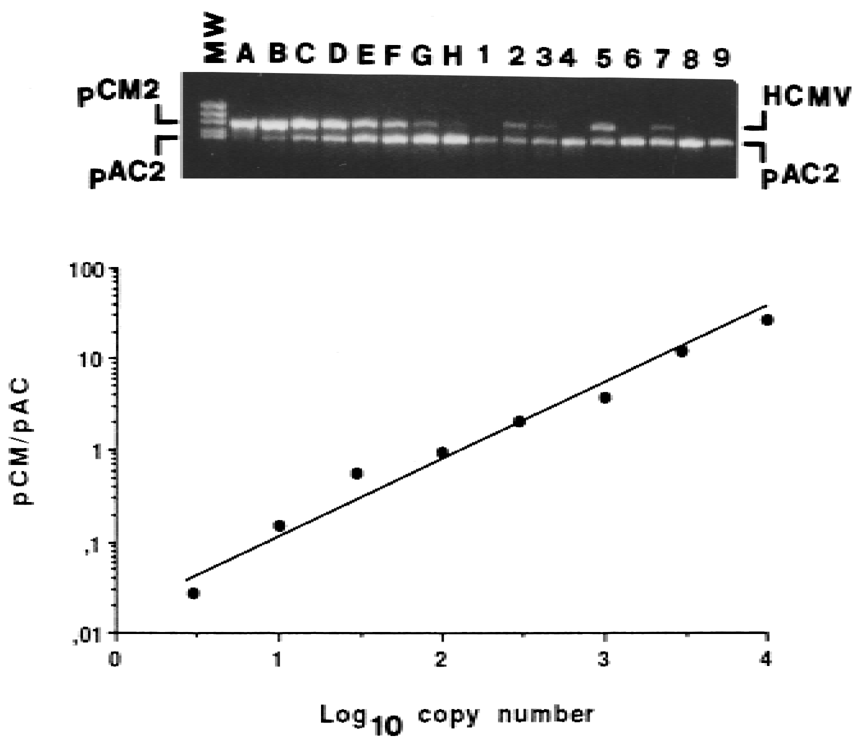

Fig. 1. Construction of the standard curve. In the upper portion of the figure, gels signals from external standards (pCM2 copy number: A, 10,000; B, 3,000; C, 1,000; D, 300; E, 100; F, 30; G, 10 and H, 3) as well as from HCMV IE1 DNA of 9 PBL samples (lines 1–9) amplified in the presence of 100 pAC2 copies (internal control), are shown. MW, molecular weight markers: pBR322 digested with Hae III (from the top to the bottom 587, 540, 504, 458, 434, 267, 234, 213 bp). In the lower portion of the figure, the standard curve obtained by densitometric analysis of gel signals is shown. HCMV DNA quantification in positive PBL samples was expressed in genome equivalents (GE) and was obtained by interpolation of HCMV/pAC2 values from the standard curve (sample # 2, 63 GE; #3, 10 GE; #5, 89 GE; #7, 18 GE).

sequences, PCR product from samples negative for DNA but competent for amplification, thus containing pAC2 PCR product, could be submitted directly to the second (nested) step of amplification using the inner primer set [Revello et al., 1995]. This method allowed reproducible HCMV DNA quantification in the range of 10–10,000 genome equivalents (GE) by using the single step quantitative PCR (Fig. 1), whereas samples containing 1–10 GE could be detected by the nested PCR protocol and were assigned an arbitrary number of 5 GE. When viral DNA levels were higher than the upper limit of the quantification system, samples were diluted to be included in the linearity range of the curve.

Antiviral Susceptibility Testing

Antiviral drug susceptibility was carried out by both the conventional [Gerna et al., 1992a] and the rapid 4-day [Gerna et al., 1995] immediate-early antigen plaque-reduction assay. Due to the slow viral growth of foscarnet-resistant HCMV strains [Baldanti et al., 1996], the plaque reduction assay for HCMV strains suspected to be foscarnet-resistant extended over 6 days instead of 4 days. HCMV strains were considered resistant when ganciclovir or foscarnet ID_{50} was at least 5 times greater than the mean ID_{50} (2.9 ± 2.1 μM for ganciclovir, and 69.9 ± 28.2 μM for foscarnet) of a series of sensitive strains, respectively [Gerna et al., 1995].

Statistical Analysis

The correlation between the number of circulating cytomegalic endothelial cells and levels of viremia, antigenemia, L- and P-DNAemia was determined by linear regression analysis. In addition, the Wilcoxon signed rank test was used for comparing mean levels of cytomegalic endothelial cells, viremia, antigenemia, L- and P-DNAemia prior to and after 1–2 weeks of antiviral treatment in patients with increase/persistence of circulating cytomegalic endothelial cells after initiation of therapy. Differences in proportions were tested by the chi-square test. All tests were two-tailed.

RESULTS

Prevalence of Circulating Cytomegalic Endothelial Cells in the AIDS Population

Thirty-two of the 1,099 (2.9%) patients examined were found to have circulating cytomegalic endothelial cells on a single or multiple occasions (Fig. 2). The incidence rose to 6.5% if calculated on the total of 489 AIDS patients positive for HCMV in blood. When considering buffy coat samples examined in the same period from the same patients, 833/1,888 (44.1%) were

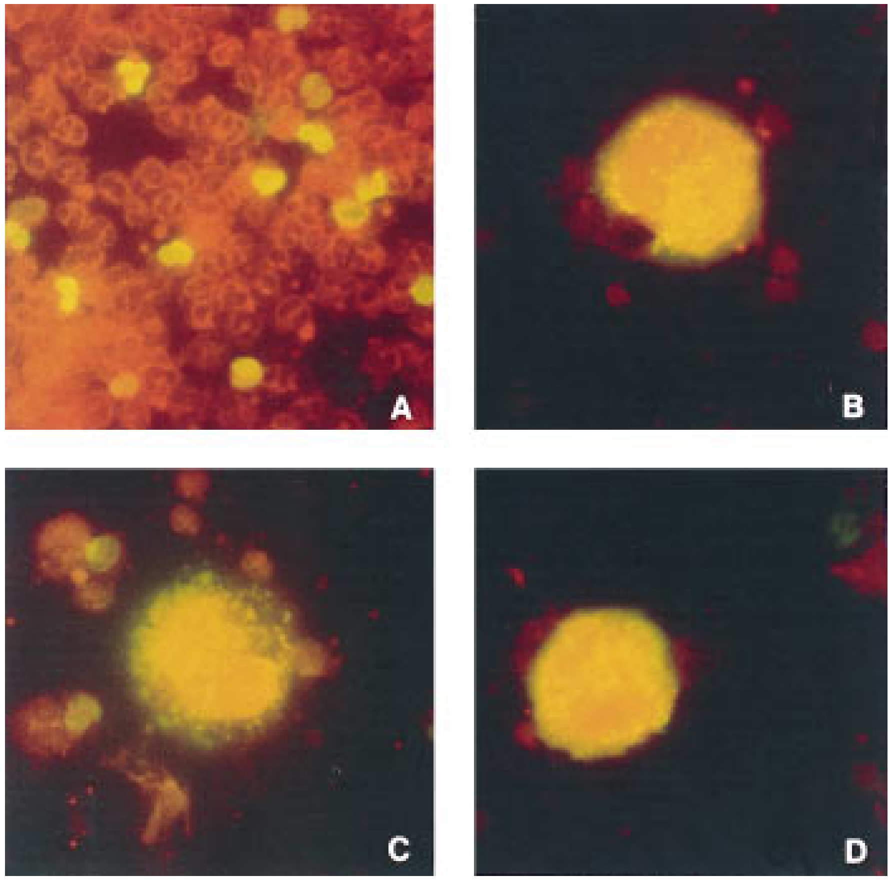

Fig. 2. Morphological aspects of (A) high level HCMV pp65-antigenemia, and (B–D) 3 circulating cytomegalic endothelial cells in patient 19 (Table 1) following emergence of ganciclovir-resistance (see Fig. 3 C). A, buffy coat; B–D, Ficoll-separated mononuclear cell fraction; ×1,000.

positive for antigenemia and 312/1,862 (16.8%) for viremia, whereas only 57/1,888 (3.0%) were positive for cytomegalic endothelial cells. When the distribution was analysed by levels of HCMV antigenemia, the incidence of cytomegalic endothelial cells was found to have increased progressively with increasing levels of antigenemia (Fig. 3). In parallel, increasing levels of HCMV viremia were associated with increasing presence of circulating cytomegalic endothelial cells. It was also observed that circulating cytomegalic endothelial cells were present in 27/68 (39.7%) and in 15/31 (48.4%) buffy coat samples showing the highest levels of antigenemia (>1,000) and viremia (>100), respectively (Fig. 3), and in 50/218 (22.9%) and in 34/121 (28.1%) samples with levels of antigenemia and viremia >100 and >10, respectively. In addition, end organ disease was present in 1.3% (8/615) of samples from patients with antigenemia levels <100, in 46.0% (69/150) of blood samples from patients with an antigenemia level of 100–1,000 and in 76.4% (52/68) of samples from patients with an antigenemia level >1,000.

Since an association between cytomegalic endothelial cells and HCMV organ involvement was suggested previously [Percivalle et al., 1993], this was investigated by stratifying samples from patients with cytomegalic endothelial cells and/or end organ disease according to the level of antigenemia. The first level was defined by antigenemia range of 100–1,000 and the second level by antigenemia level >1,000 (Fig. 4). It was found that, according to the antigenemia level, while the incidence of cytomegalic endothelial cells in the absence of end organ disease (10.7% vs. 10.3%, respectively) and that of end organ disease in the absence of cytomegalic endothelial cells (40.8% vs. 48.9%, respectively) did not vary significantly, the incidence of end organ disease associated with cytomegalic endothelial cells increased significantly. In fact, while in samples with antigenemia of 100–1,000, end organ disease appeared to be only associated sporadically with to cytomegalic endothelial cells (9/150, 6% of samples), in samples with antigenemia >1,000 a much higher proportion of end organ disease (19/68, 28% of cases) were cytomegalic endothelial cell-associated ($P < 0.0001$).

Positive and negative predictive values (ppv and npv) of cytomegalic endothelial cell detection for diagnosis of HCMV end organ disease were 54.9% and 44.3% with antigenemia of >100, and 73.1% and 21.4% with antigenemia of >1,000, respectively. On the other hand, antigenemia had a ppv of 55.5% and a npv of 98.7% using a cutoff of 100, and a ppv of 76.4% and a

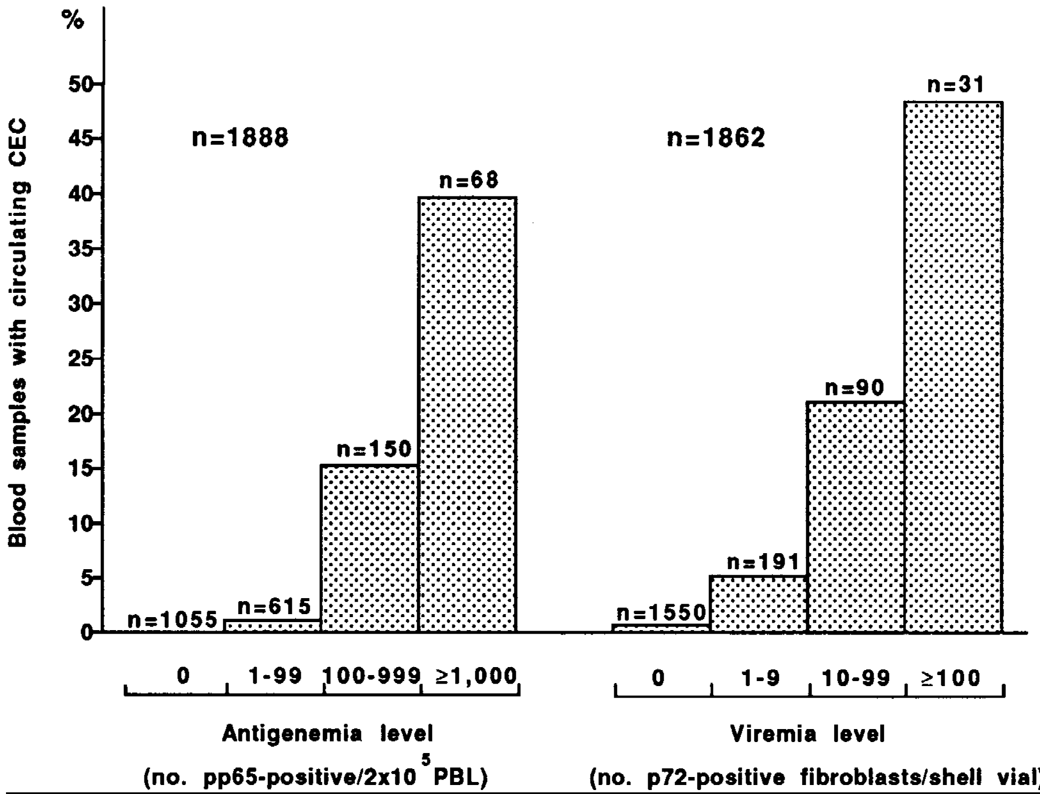

Fig. 3. Distribution of circulating cytomegalic endothelial cells according to the antigenemia and viremia level in blood samples from 1,099 AIDS patients examined for HCMV load in 1994–1995.

npv of 89.9% with a cutoff of 1,000. Viremia showed a ppv of 52.1% and a npv of 97.4% with a cutoff of 10, whereas with a cutoff of 100 ppv was 74.2%, and npv was 84.0%.

Correlation of Circulating Cytomegalic Endothelial Cells and HCMV Load in Blood

A selected group of 38 AIDS patients with HCMV disease with single or multiple episodes of circulating cytomegalic endothelial cells during follow-up was investigated to study the correlation with HCMV load and end organ disease. The main clinical, therapeutic and virological characteristics of these patients are shown in Table I concomitantly with the primary cytomegalic endothelial cell episode and with the clinical conditions associated with cytomegalic endothelial cell appearance. Of the 38 patients, 18 had circulating cytomegalic endothelial cells on a single occasion, whereas in 20 patients circulating cytomegalic endothelial cells were detected on several occasions (n = 2–7) during follow-up. The mean time of follow-up was 157 days with a range of 5–730 days. The overall number of cytomegalic endothelial cells episodes observed in these patients during follow-up was 75. However, in Table I besides the primary cytomegalic endothelial cell episodes, a few additional secondary cytomegalic endothelial cells episodes (2 or 3) are shwon in only 3 patients (no. 3, 4 and 19), in whom sequential cytomegalic endothelial cell episodes were associated with different clinical conditions compared to the primary episode. Thus, on the whole, in Table I, 43 cytomegalic endothelial cell episodes from 38 patients are shown.

In the group of 43 cytomegalic endothelial cell episodes shown in Table I, the median cytomegalic endothelial cell number was $2.0/2 \times 10^5$ PBL examined. In addition, the median level of HCMV viremia was 40 p72-positive cultured fibroblasts/2×10^5 PBL inoculated into a shell vial (range 0–1,000). Levels of viremia were high (10–100) or very high (100–1,000) in 20 and 17 episodes of circulating cytomegalic endothelial cells reported in Table I, respectively, raising the total number of episodes associated with high viremia level to 37/43 (86.0%). Similarly, the median level of pp65-antigenemia was 700 pp65-positive/2×10^5 PBL examined (range 54–5,000). Levels of pp65-antigenemia were high (100–1,000) or very high (1,000–10,000) in 23 and 18 episodes, respectively, bringing the overall number of episodes associated with high level antigenemia to 41/43 (95.3%). Finally, the median levels of L-DNAemia and P-DNAemia were 18,000 and 1,995 GE/1×10^5 PBL examined (range 446–524,807 and 5–63,095), respectively. Levels of L-DNAemia were high (1,000–10,000 GE/1×10^5 PBL) or very high (10,000 DNA GE) in 25 and 13 episodes, respectively, with an overall number of episodes with a high L-DNAemia level of 38/41 (92.7%). Levels of P-DNAemia were high or very high (according to the same criteria) in 17/29 (58.6%) patients with cytomegalic endothelial cells. In

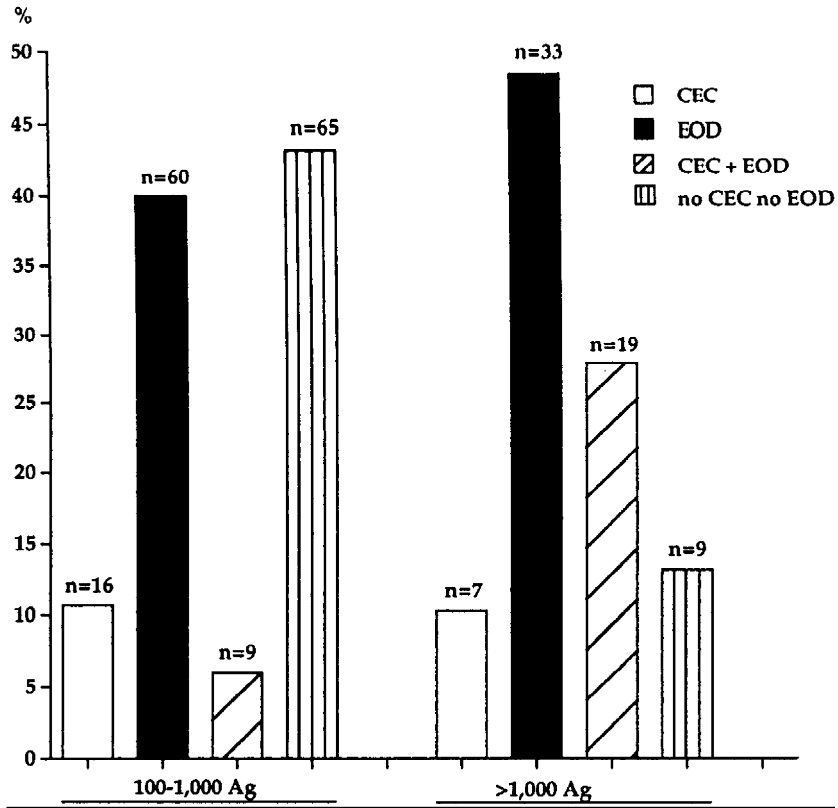

Fig. 4. Distribution of circulating cytomegalic endothelial cells (CEC) and end-organ disease (EOD) in 2 groups of AIDS patients stratified by levels of antigenemia of 100–1,000 and >1,000.

addition, 32/38 patients (84.2%) had an overt HCMV end organ disease. However, in 20/38 (52.6%) patients who were followed-up for >4 months, the presence of end organ disease preceded consistently cytomegalic endothelial cell appearance by at least 3 months.

In individual patients the highest number of circulating cytomegalic endothelial cells was not always associated with the highest levels of viremia, antigenemia, L-DNAemia. There was no significant correlation between the number of circulating cytomegalic endothelial cells and levels of viremia, antigenemia and L-DNAemia. On the other hand, a highly significant correlation was found between number of cytomegalic endothelial cells and P-DNAemia levels ($r = 0.699$; $P < 0.0001$). In parallel, a significant correlation was found between levels of antigenemia and P-DNAemia ($r = 0.651$; $P = 0.0001$) and between levels of L-DNAemia and P-DNAemia ($r = 0.723$; $P < 0.0001$).

Clinical Conditions Associated with Appearance of Circulating Cytomegalic Endothelial Cells

In the 38 AIDS patients shown in Table I the primary cytomegalic endothelial cell episode was found to be associated with one of the following conditions: i) lack of anti-HCMV treatment (31 patients); ii) insufficient anti-HCMV treatment (patients 3 and 8); iii) emergence of ganciclovir- or ganciclovir- and foscarnet-resistant HCMV strains (patients 1 and 19); iv) recent antiviral treatment (patients 11, 29 and 33). The secondary cytomegalic endothelial cell episodes shown in Table I were due to lack of treatment (patient 19), emergence of ganciclovir-(patients 3 and 4) or ganciclovir- and foscarnet-double resistance (patient 3) or response to treatment (patient 19). On the whole, of the 75 cytomegalic endothelial cell episodes relevant to the 38 patients of Table I, 42 (56.0%) were associated with lack of treatment, 17 (22.7%) with short-term response to antiviral treatment, 13 (17.3%) with 5 HCMV drug-resistant strains and 3 (4.0%) with insufficient treatment.

Long-term Follow-up of 4 AIDS Patients with Respect to Circulating Cytomegalic Endothelial Cells

The follow-up analysis of the 4 AIDS patients shown in Figure 5 exemplifies some of the clinical conditions which are associated mostly with cytomegalic endothelial cell appearance (cytomegalic endothelial cell number is indicated by circled numbers). In patient A (no. 32, Table I) multiple episodes of cytomegalic endothelial cell appearance, occurring after more than 6 months of follow-up, were associated with lack of specific antiviral treatment, appearance of fever and peak values of all measured viral parameters, whereas initiation of treatment with either ganciclovir or both gan-

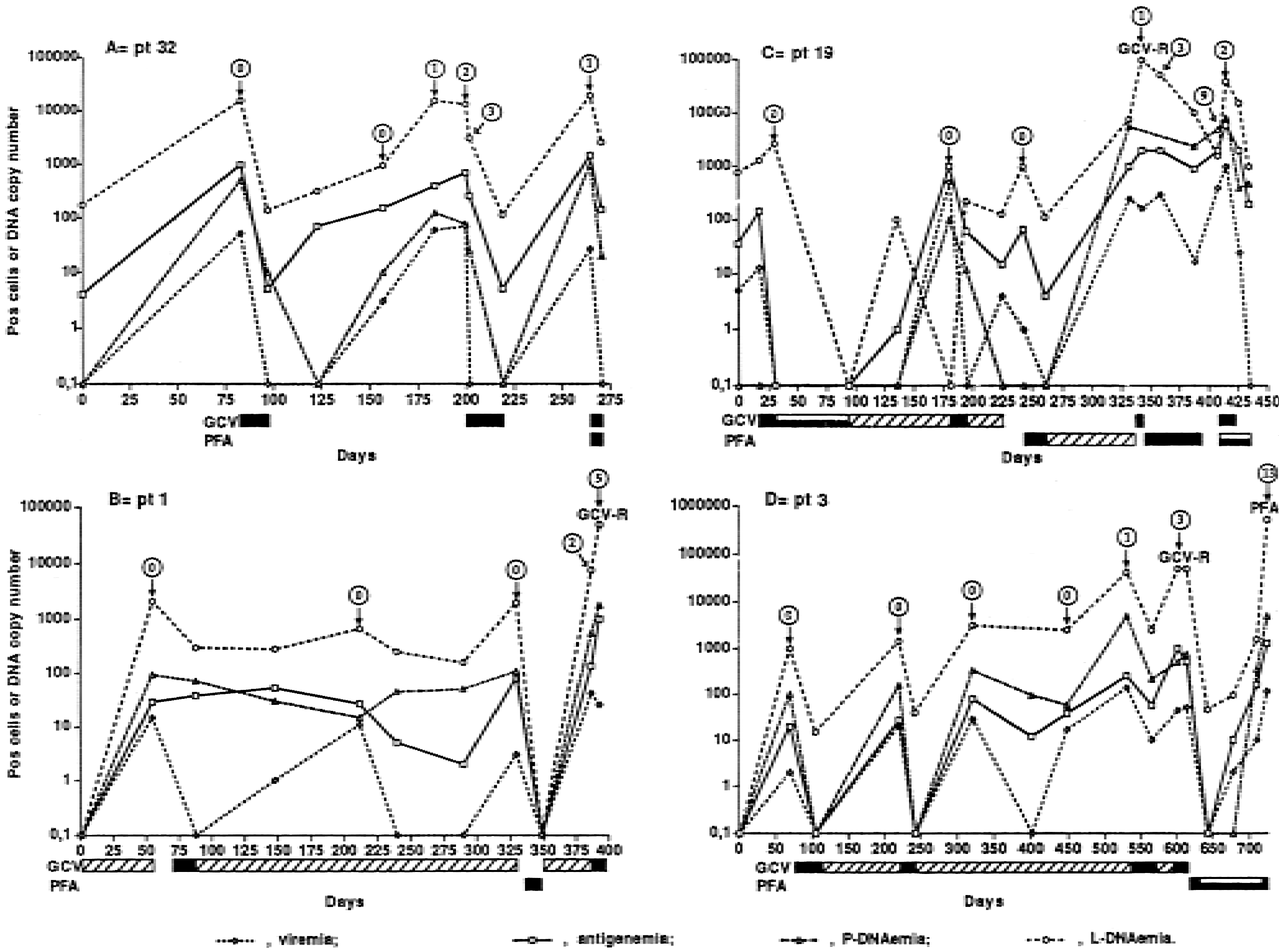

Fig. 5. Virologic follow-up of 4 AIDS patients. Circled numbers indicate number of circulating cytomegalic endothelial cells detected in blood collected at the indicated time. Circled numbers with "zero" indicate cytomegalic endothelial cells absence on cytospin preparations of mononuclear cells. Absence of circled number means absence of cytomegalic endothelial cells on routine cytospin preparations of buffy coat (PBL). GCV-R, ganciclovir-resistance; PFA-R, foscarnet-resistance. Closed, semiclosed or dashed bars indicate induction, maintenance or suboptimal maintenance doses of the indicated drug, respectively.

ciclovir and foscarnet caused rapid disappearance of both cytomegalic endothelial cells and fever along with drop in level of viral markers. In patients B and C (no. 1 and 19, Table I), cytomegalic endothelial cells appeared about a year after diagnosis of HCMV retinitis and were related to the emergence of a ganciclovir-resistant strain isolated from blood of both patients. In both patients cytomegalic endothelial cells were associated with peak levels of viremia, antigenemia, L- and P-DNAemia. In addition, resistance appeared after several months of treatment of both patients with induction and maintenance courses of ganciclovir. Finally, in patient D (no. 3, Table I), cytomegalic endothelial cells appeared in blood nearly a year after diagnosis of a laryngeal localization of HCMV disease and was followed by the emergence of the ganciclovir-resistance following a year of maintenance ganciclovir treatment. Initiation of foscarnet treatment strikingly reduced levels of all viral markers with cytomegalic endothelial cells disappearance and amelioration of clinical symptoms. However, 3 months of maintenance treatment with foscarnet caused the emergence of foscarnet-resistance which was associated with a relapse of HCMV laryngitis and a high number of circulating cytomegalic endothelial cells as well as a high viral load. The same HCMV strain was shown to share both ganciclovir- and foscarnet-resistance [Baldanti et al., 1995; Sarasini et al., 1995].

Appearance/Increase/Persistence of Circulating Cytomegalic Endothelial Cells in AIDS Patients after Initiation of Antiviral Treatment

In 11 (28.9%) of the 38 AIDS patients reported in Table I, a "paradox" phenomenon was observed consisting of appearance, increase in number or persistence of circulating cytomegalic endothelial cells in blood of treated patients during the first (and, sometimes, also the second) week of treatment with ganciclovir. In detail, in 4 patients cytomegalic endothelial cells appeared during ganciclovir treatment, in 4 additional patients increased in number and in 3 patients number

TABLE II. Increase/Persistence of Circulating CEC in AIDS Patients With Disseminated HCMV Infection Following Initiation of Antiviral Treatment With Ganciclovir

Patient number	Ag	Vir	CEC	L-DNA/P-DNA	Antiviral treatment (days)
6.	3,000	270	6	137,153/11,220	−1
	1,300	3	77	156,234/19,952	+7
	ND	2	0	ND/8,910	+11
7.	3,000	250	3	31,622/7,940	0
	1,000	6	3	19,952/1,412	+7
	25	0	0	100/251	+15
	0	0	0	30/15	+22
10.	600	103	1	1,258/ND	−1
	86	0	1	630/16	+7
	8	0	0	ND/ND	+16
	0	0	0	316/<10	+23
11.	1,500	10	0	ND/ND	−1
	2,000	10	3	7,943/44	+12
	310	0	0	ND/neg	+26
	0	0	0	5[a]/neg	+31
13.	1,000	600	1	50,118/3,981	−1
	1,100	13	2	35,481/707	+1
	600	5	4	56,234/1,258	+7
	39	0	1	ND/158	+13
	2	0	0	1,316/neg	+20
14.	900	57	1	63,095/12,590	−3
	177	0	11	25,118/13,000	+3
	4	0	0	251/3,162	+8
15.	298	13	0	2,500/145	−5
	500	26	1	5,689/251	−2
	109	0	1	500/57	+8
	0	0	0	5[a]/neg	+10
16.	400	90	0	ND/2,818	−1
	65	0	2	2,818/17	+9
	9	0	0	1,584/neg	+29
17.	385	13	4	10,000/501	−6
	300	2	8	50,118/125	+7
	130	1	0	14,125/33	+13
	35	0	0	445/56	+16
18.	246	27	0	3,981/97	−12
	48	0	2	1,445/5[a]	+7
29.	2,000	1,000	0	125,800/12,589	−4
	200	26	10	79,000/7,585	+10
	150	3	2	12,589/3,981	+24

[a]See footnote [a] of Table 1.

remained stable. In the group of 11 AIDS patients reported in Table II the mean cytomegalic endothelial cell number was 1.5 (range 0–6) prior to initiation of treatment and 11.1 (range 1–77) after a mean treatment duration of 7.6 (range 3–12) days ($P = 0.011$), when the mean cytomegalic endothelial cell number reached the peak. In the same group of patients, this significant increase in cytomegalic endothelial cell number was associated with a significant decrease ($P = 0.016$) in mean level of antigenemia from 1,230 (range 246–3,000) to 535 (range 48–2,000). Similarly, the fall in mean level of viremia from 222.3 (range 10–1,000) to 3.8 (range 0–26) was statistically significant ($P = 0.003$), whereas mean levels of L-DNAemia and P-DNAemia were not significantly different prior to (47,581 and 5,776 GE, respectively) and after (43,175 and 4,823 GE, respectively) the short mean treatment time of 7.6 days. The mean time to cytomegalic endothelial cell disappearance was 16.4 (range 8–29) days.

DISCUSSION

The main objective of this study was to define the epidemiological, clinical, diagnostic, and prognostic significance of cytomegalic endothelial cells in AIDS patients. From an epidemiological standpoint, the incidence of cytomegalic endothelial cell detection was shown to be low both in the AIDS population (2.9%) and in the fraction of the AIDS population presenting HCMV in blood (6.5%). However, the incidence of cytomegalic endothelial cells increased to 22.9% and 28.1% in blood samples with levels of antigenemia and viremia >100 and >10, respectively. On the other hand, in the selected cytomegalic endothelial cell-positive fraction of the AIDS population, cytomegalic endothelial cells were associated with high levels of antigenemia (>100) and viremia (>10) in 95.3% and 86.0% of cytomegalic endothelial cells episodes, respectively. Similarly, the association proposed originally between cytomegalic endothelial cells and HCMV organ localizations was found to be nearly 30% in the aliquot of the AIDS population with an antigenemia level >1,000, whereas it was 84% in the selected cytomegalic endothelial cell population. Thus, while in the AIDS population high levels of viremia and antigenemia as well as presence of HCMV end organ disease were associated to cytomegalic endothelial cells in <50% of patients examined, in the selected cytomegalic endothelial cell population such an association rose to >80%. This difference was likely to be attributable to the longer follow-up in the selected compared to the general AIDS population.

A detailed analysis of the results seems to justify the conclusion that circulating cytomegalic endothelial cells correlate with high viral load and duration of end organ disease in AIDS patients with late-stage disseminated HCMV disease. This conclusion is based on the following findings: i) in the general AIDS population cytomegalic endothelial cell incidence increases in parallel with increasing levels of antigenemia; ii) in the majority of the 38 AIDS patients undergoing follow-up cytomegalic endothelial cells were detected at least 3 months after appearance of end organ disease; iii) in two previous studies on HCMV retinitis and gastrointestinal disease in AIDS patients cytomegalic endothelial cell incidence was found to be of only 9/99 (9.1%) and 2/29 (6.9%) patients, respectively, upon first end organ disease detection, in the presence of a mean antigenemia level of about 100 [Gerna et al., 1994a; Gerna et al., 1997]; iv) in 3/4 patients reported in Figure 3 cytomegalic endothelial cells were detected about a year after end organ disease appearance concomitantly with a very high viral load. Thus, cytomegalic endothelial cells appear to be a marker of late-stage disseminated HCMV disease in addition to high levels of viremia, antigenemia, L-DNAemia and P-DNAemia.

In the selected group of the 38 AIDS patients in an advanced stage of the disease (32 had HCMV end organ disease and 6 disseminated HCMV disease), cytome-

galic endothelial cell presence was associated consistently with one of the following clinical conditions: lack of specific anti-HCMV treatment; insufficient maintenance treatment; emergence of anti-HCMV drug resistance; or recent onset of specific antiviral treatment. In addition to the first two conditions, this study has demonstrated that two other major conditions were associated with cytomegalic endothelial cell appearance: antiviral drug resistance, following prolonged periods of treatment with either ganciclovir or foscarnet (17.3%); and short-term response to antiviral treatment (22.7%). In AIDS patients responding to anti-HCMV treatment, cytomegalic endothelial cell disappearance from blood occurred mostly in 1–2 weeks [Gerna et al., 1993]. On the other hand, in patients with proven ganciclovir- or ganciclovir- and foscarnet-resistance, cytomegalic endothelial cell appearance was the rule (See patient B, C and D of Fig. 1) and was associated with the highest levels of viremia, antigenemia, L- and P-DNAemia. This close association between antiviral drug resistance and presence of circulating cytomegalic endothelial cells confirms that cytomegalic endothelial cells appear in blood only in late stages of a disseminated HCMV disease, when viral load reaches the highest peak. In our experience, prolonged (several months) antiviral treatment preceded consistently the emergence of the drug-resistance [Sarasini et al., 1995].

The observation that in some patients cytomegalic endothelial cells appeared, increased in number or persisted after 1–2 weeks of effective antiviral treatment remains to be explained. Concomitantly, levels of pp65-antigenemia and viremia fell significantly, whereas levels of L- and P-DNAemia did not change significantly in the same short lapse of time. The explanation of such a paradox phenomenon may reside in the fact that, in the first week of treatment, although viral DNA replication is blocked, the endothelium tends to clear HCMV from the vascular tree by detaching cytomegalic endothelial cells from the vessel wall to reconstitute the continuity of normal endothelium. From a clinical standpoint, the increase in number of circulating cytomegalic endothelial cells during the first 1–2 weeks of treatment must not suggest shifting to an alternative drug, when there is a concomitant and dramatic drop in viremia and antigenemia levels, while L- and P-DNAemia persist at a stable level.

In conclusion, cytomegalic endothelial cells are associated with high HCMV load in AIDS patients with late-stage HCMV disease and may be associated to long-lasting end organ disease. Cytomegalic endothelial cells presence in blood is mostly caused by untreated long-lasting HCMV infection or emergence of drug-resistance and may be considered an additional marker of advanced HCMV disease.

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